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Serum Retinol, Iron Status and Body Composition of Lactating Women in Nandi, Kenya

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Key Words

Hemoglobin · Hematocrit · Serum ferritin · Vitamin A status · Fat free mass · Fat mass · Breast-feeding · Breast milk fat

Abstract

Background: Maternal vitamin A and iron status was investigated among lactating mothers in a rural community in Kenya. The aim of the study was to establish the prevalence and the relationship of these key nutrients to maternal body composition. **Methods:** Eighty-eight mothers provided samples of breast milk and blood for determination of breast milk vitamin A, serum retinol and ferritin. Estimators of body composition were based on the mother's weight, height and skinfold measurements. **Results:** A total of 78.1% women had breast milk retinol <1.05 µmol/l with 38 and 62% having lactated for a period of <4 and >4 months, respectively. Prevalence of severely deficient serum retinol <0.35 µmol/l and ferritin <12 µg/l was 10 and 37%, respectively. Women with serum ferritin <12 µg/l had significantly lower average hemoglobin ($p < 0.01$), hematocrit ($p < 0.01$) and serum retinol ($p < 0.05$). Serum retinol of mothers who had lactated for <4 months was significantly but negatively correlated with total body fat ($r = -0.40$; $p < 0.05$). With a lactation period of >4 months a close relationship was found between serum retinol and hemoglobin ($r = 0.26$;

$p < 0.01$), serum retinol and serum ferritin ($r = 0.20$; $p < 0.05$), and fat free mass significantly but negatively correlated with breast milk fat ($r = -0.27$; $p < 0.05$). Serum retinol in combination with hematocrit significantly affected both maternal hemoglobin ($p < 0.01$) and serum ferritin ($p < 0.01$). **Conclusion:** A high prevalence of vitamin A and iron deficiency was observed in this group of lactating women. Low levels of fat mass were directly related to these indicators of malnutrition.

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Introduction

Vitamin A and iron deficiency anemia are considered to be among the major nutritional deficiencies in developing countries [1, 2]. Low weight in combination with small iron stores and low serum retinol levels leads to a risk of depletion and hence malnutrition. Furthermore the additional low concentrations of both serum and breast milk vitamin A put the solely breast-fed child at high risk of low intake of vitamin A [3]. In the national survey of preschool-age children 35% of this vulnerable group were reported to be vitamin A-deficient [4]. The only small survey done in Machakos indicated that vitamin A deficiency during pregnancy is community-specific [5]. National figures for iron deficiency are not yet available. A survey done on pregnant women has reported 57%

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to be anemic [6]. The lactating mother in Kenya has been found to be underweight while living under the constraints of a limited food supply and the demands of hard physical work [7, 8]

Successive pregnancies and lactation have cumulative effects on specific nutrients [9] and depending on maternal body stores lead to the well-documented maternal depletion syndrome [10]. The key nutrients affected may include vitamin A and iron, whose requirements are increased during pregnancy and lactation. Vitamin A deficiency may exacerbate iron deficiency anemia [11], and its supplementation to pregnant mothers [12] and children [13] has improved iron status as well as child health [14].

The national survey on household food security reports that 23% of Kenyans come from households where members of the family are unlikely to meet their minimum energy requirements even if the household concentrated all its spending on food [15]. In these households women frequently enter pregnancy with little or no maternal fat stores [16]. Vitamin A and iron may be a problem of public health significance among all the members of the family including the lactating mothers. Maternal nutrition is of paramount importance if the nutrition and health condition of the child are to be improved [17]. Supplementation with iron or vitamin A tends to target the pregnancy phase of the reproductive cycle. Relying on intervention in the late first trimester or early second trimester of pregnancy may be too late [18]. Information on how and when to intervene is critical and needs to be tailored to meet the unique needs of each target group [19, 20]. This necessitates the identification of communities where both pregnant and lactating mothers may not only be undernourished but are also at risk of vitamin A and iron deficiency.

The present study was therefore undertaken to investigate the prevalence of vitamin A and iron deficiency anemia among lactating mothers living in a rural community in Kenya. We present the results of a cross-sectional study in which we determined the prevalence of vitamin A and iron deficiency in lactating women in the Kokwet location of Nandi district, Kenya. We also examined whether there is an association between serum retinol, ferritin, breast milk retinol concentrations and body composition.

Materials and Methods

The study was carried out in December 1998 and January 1999. It involved the examination of 88 normal lactating women aged 15–45 years with their breast-feeding children aged between 2 weeks and

15 months from 7 villages in Kokwet location of Nandi district, an area 30 km southwest of Eldoret Town. The women in this study came from a rural farming community and are considered at risk in relation to low energy and nutrient intake. All the lactating mothers in the 7 villages were identified and visited in their homes and interviewed. In addition to food intake, child health and nutritional status, other variables of interest were maternal body composition, iron and vitamin A status. The study was carried out following the ethical standards of Moi University, Eldoret and with clearance and permission from the Kenyan government.

Anthropometry, Blood and Breast Milk Collection

Information on age, parity, length of lactation, and interval since last pregnancy was recorded by using a questionnaire. Anthropometric measurements comprising height, weight, mid-upper arm circumference and skinfold thickness were performed and used to determine maternal fat mass and fat free mass [21]. Venous blood samples of about 5 ml were taken and stored on ice for transportation to the laboratory. Serum was separated from blood by centrifugation at 200 g for 15 min at room temperature on arrival and samples stored separately at -20°C in the dark. Breast milk was collected from one breast that had not been used to feed the infant for at least 1 h [22] and covered and stored in a cool box for transportation to the laboratory. Milk samples were re-homogenized at room temperature and the samples stored separately at -20°C in the dark.

Biochemical Analysis

Leishman's stain was used to make blood smears, and erythrocyte and leukocyte status of the lactating mothers was determined and cell morphology classified into three categories of normal, mild and moderate. The blood slides were examined for the distribution of macrocytic cells due to vitamin B_{12} or folic acid deficiency, microcytic hypochromic anemia due to iron deficiency and the presence of hypochromic cells. A Coulter counter, model 560 was used to determine hemoglobin (Hb), hematocrit ratio (Hct), mean cell volume (MCV) and red blood cell (RBC) count.

The creatamocrit method as described by Lucas et al. [23] was used to determine breast milk fat and energy concentration: The vitamin A concentration was expressed as a ratio to the fat concentration in percent, i.e. $1.5\text{ }\mu\text{mol/l}$ vitamin A: 5% fat = $0.3\text{ }\mu\text{mol}/\%$ fat [20, 22]. Retinol levels in serum and breast milk were assayed using high-performance liquid chromatography. Measurement of serum ferritin (SF) was performed using the enzyme-linked immunosorbent assay (ELISA, Boehringer Mannheim Immundiagnostik, Mannheim, Germany).

Nutritional anemia was assessed by using both Hb and Hct [24]. The lactating mothers were categorized as having iron deficiency anemia when found with a Hb of $<12\text{ g/dl}$ or a Hct ratio of <37 . Depletion of iron stores was based on the levels of SF concentration: severe $<12\text{ }\mu\text{g/l}$; marginal $12\text{--}29\text{ }\mu\text{g/l}$ and normal $\geq 30\text{ }\mu\text{g/l}$. Vitamin A status was based on serum retinol concentration: deficient, $<0.35\text{ }\mu\text{mol/l}$ ($10\text{ }\mu\text{g/dl}$) and marginal, $<0.70\text{ }\mu\text{mol/l}$ ($20\text{ }\mu\text{g/dl}$). The same cutoff points were used for deficiency in breast milk retinol levels.

Statistical Analyses

Data were analyzed with the SPSS/PC statistical package (version 9.2 1998). Mean, standard deviations and median were calculated. Independent samples' Student's *t* test was used to determine the significance of the differences between lactating mothers with normal

Table 1. Characteristics and biochemical indexes of the lactating women

	Percentiles			
	Mean \pm SD	5th	50th	95th
Mother's age, years	27.2 \pm 6.1	18.8	28.0	38.3
Child's age, months	7.6 \pm 4.9	1.3	6.2	15.0
Child's birth weight	3.4 \pm 0.54	2.5	3.4	4.2
Children	3.9 \pm 2.4	1.0	3.5	8.5
Lactation length, months	7.8 \pm 4.9	1.3	8.2	15.0
Height, cm	161.1 \pm 5.9	150.9	161.9	171.6
Weight, kg	56.5 \pm 9.7	42.5	55.0	76.9
Mid arm circumference	24.8 \pm 2.7	21.0	24.7	29.8
Body mass index	21.7 \pm 3.2	17.2	21.4	28.0
Body fat, %	26.1 \pm 5.4	7.4	26.1	35.9
Fat free mass, kg	41.3 \pm 5.1	33.3	41.3	50.6
Hb, g/dl (n = 73)	11.9 \pm 1.8	8.9	12.3	14.8
Hct ratio (n = 73)	34.9 \pm 3.2	28.7	36.0	38.8
MCV/l (n = 69)	81 \pm 7.5	67.0	83.0	95.0
RBC, 10 ¹² /l (n = 56)	44.6 \pm 3.7	39.1	44.3	52.5
SF, μ g/l (n = 67)	21.5 \pm 16.3	4.5	15.4	50.1
Serum retinol, μ mol/l (n = 69)	0.69 \pm 0.26	0.34	0.69	1.2
Breast milk fat, % (n = 68)	4.3 \pm 2.3	1.0	4.0	9.6
Breast milk retinol, μ mol/l (n = 73)	0.90 \pm 0.7	0.23	0.70	2.84
Geometric mean \pm SD, n = 88 except where otherwise noted.				

Table 2. Significance of mean values of micronutrients and body composition in lactating mothers with normal and depleted iron stores based on SF levels

Variable	Iron stores ^a	n	Mean \pm SD	p value ^b
Hb, g/dl	severe depletion	25	10.7 \pm 1.8	<0.01
	marginal to normal	42	12.6 \pm 1.4	
Hct ratio	severe depletion	25	32.6 \pm 3.6	<0.01
	marginal to normal	42	36.3 \pm 2.1	
Serum retinol, μ mol/l	severe depletion	24	0.63 \pm 0.15	<0.05
	marginal to normal	41	0.74 \pm 0.30	
Breast milk retinol, μ mol/l	severe depletion	25	0.98 \pm 0.90	>0.05
	marginal to normal	42	0.86 \pm 0.60	
Body mass index	severe depletion	25	22.3 \pm 2.6	>0.05
	marginal to normal	42	21.4 \pm 3.7	
% body fat	severe depletion	25	27.5 \pm 5.3	>0.05
	marginal to normal	42	25.3 \pm 5.8	
Fat free mass, kg	severe depletion	25	42.0 \pm 4.1	>0.05
	marginal to normal	42	40.8 \pm 6.1	

^a SF < 12 μ g/l = severe depletion; SF > 12 μ g/l = marginal to normal.

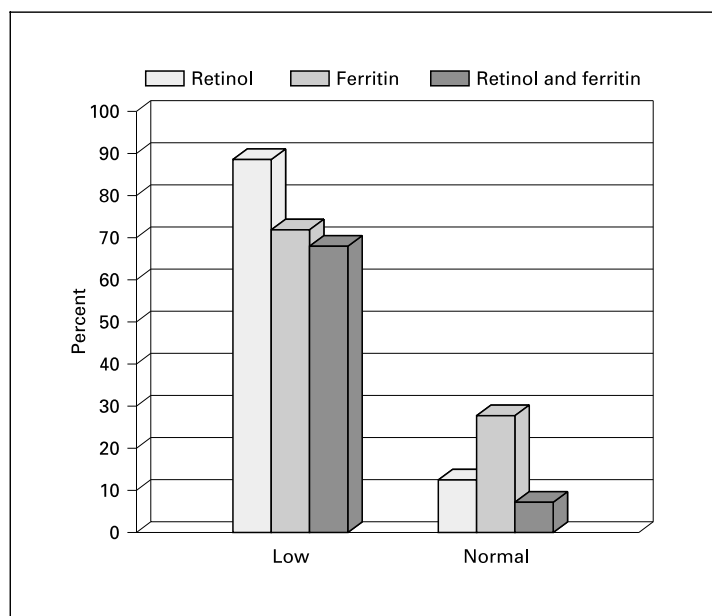
^b p value based on t test for independent samples.

and depleted iron stores. The chi-square test was used to compare the difference between proportions of mothers with normal and low serum retinol and ferritin. Pearson's correlation tests were performed to examine the relationships between body composition, serum retinol and measures of iron status. In order to identify individual factors and the combination of factors likely to affect fat stores, iron deficiency anemia and the vitamin A status of the rural lactating

mothers, both backward and step multiple regressions were done. Hb status, SF and percent body fat were treated as dependent variables. Due to a skewed distribution natural log-transformed values were used for SF concentration.

The following predictor variables were used in the multiple regression analyses: maternal: age and parity; child: age and reported birth weight; vitamin A: breast milk fat, breast milk retinol and

Fig. 1. Maternal iron stores and vitamin A status. Low: SF <30 µg/l, serum retinol <1.05 µmol/l. Normal: SF >30 µg/l, serum retinol >1.05 µmol/l. Pearson χ^2 $p < 0.05$.



serum retinol; anemia: RBC count, MCV, SF, Hct and Hb; body composition: mid arm circumference, body mass index, percent body fat and fat free mass. Using $p > 0.10$ for exclusion, the significant ($p < 0.01$) combination of variables related to Hb, iron and fat stores were identified.

Results

All the mothers lactating infants <15 months were recruited from 7 villages making a total of 88 mother-child pairs. From all mothers body composition measurements were taken. Eight mothers had infants of less than 1 month and 12 were unable to provide breast milk. Fifteen declined to provide blood. Table 1 shows maternal and child characteristics and indices of iron and serum retinol status. The average (\pm SD) age of the breast-feeding child was 7.6 (4.9) months. On average, percent body fat of the mothers was 26.1 (5.4), while fat free mass was 41.3 (5.1). Average breast milk retinol, serum retinol and SF were 0.90 (0.7) µmol/l, 0.69 (0.26) µmol/l and 21.5 (16.3) µg/l, respectively.

Vitamin A status of the lactating mothers based on serum retinol concentrations was 10, 40.7 and 49.3%, respectively, for severely deficient (<0.35 µmol/l), marginal (<0.70 µmol/l) and adequate (>0.70 µmol/l) status. Low breast milk retinol concentration (<1.05 µmol/l) was found in 78.1% of these lactating women. Mothers who had both low serum and breast milk retinol concentration were 55.7%.

Mild to moderate microcytosis and macrocytosis occurred in 29 and 16% of the mothers while 35% of the mothers had mild to moderate hypochromia. Hb status <12 g/dl and Hct ratio <37 were found in 43.8 and 69.9% of the women, respectively. SF concentration was severely deficient (<12 µg/l) in 37%, marginal (<30 µg/l) in 39% and adequate (>30 µg/l) in 24% of the lactating mothers.

Mothers with severely depleted iron stores (SF <12 µg/l) had significantly lower average Hb ($p < 0.01$), Hct ($p < 0.01$) and serum retinol levels ($p < 0.05$, table 2). There was a significant difference ($p < 0.05$) between mothers (67%) with low serum retinol (<1.05 µmol/l) and SF (<30 µg/l) and mothers (7.7%) with both normal serum retinol (>1.05 µmol/l) and SF (>30 µg/l) (fig. 1).

Mothers with a lactation period of <4 or >4 months were 37.7 and 62.3%, respectively. In the early months of lactation (<4 months) serum retinol was significant but negatively correlated with total body fat ($r = -0.40$; $p < 0.05$). Mothers with a longer lactation period had fat free mass that was significant but negatively correlated with breast milk fat ($r = -0.27$; $p < 0.05$). The correlation matrix relating maternal Hb status as well as SF concentration with Hct, and serum retinol is shown in table 3. Mothers with a lactation period of <4 months had a Hb status that was highly significant and positively correlated with SF ($r = 0.46$; $p < 0.01$) and Hct ($r = 0.65$; $p < 0.01$). For both Hb status and SF concentration the correlation coefficients were high and statistically significant for lactation duration of >4 months. Serum retinol was highly

Table 3. Correlation and level of statistical significance of maternal indexes of iron and serum retinol for lactation duration of <4 or >4 months

	Lactation <4 months (n = 30)				Lactation >4 months (n = 50)			
	Hb		ferritin		Hb		ferritin	
	r	p	r	p	r	p	r	p
Hb			0.46	<0.05			0.53	<0.01
Ferritin	0.46	<0.01			0.54	<0.01		
Hct	0.65	<0.01	0.43	<0.05	0.77	<0.01	0.67	<0.01
Retinol	-0.06	>0.05	0.10	>0.05	0.26	<0.01	0.20	<0.05

Table 4. Coefficients (β) and significance levels (p) from multiple regression analyses of Hb for all mothers and iron store status for mothers with ferritin concentration in serum <30 $\mu\text{g/l}$ as the dependent variables

Predictor variables	Hb status (n = 67)					Ferritin concentration (<30 $\mu\text{g/l}$) (n = 51)				
	β	r	r ²	F	p	β	r	r ²	F	p
Constant	-2.89					-22.10				
Hct	0.43	0.74	0.54	40.0	<0.01	1.03	0.56	0.32	17.4	<0.01
Constant	-4.00					-27.04				
Hct	0.42	0.77	0.60	24.7	<0.01	1.00	0.64	0.41	12.8	<0.01
Serum retinol	1.80					8.78				

significant and positively correlated to Hb status ($r = 0.26$; $p < 0.01$) and significantly and positively correlated to SF ($r = 0.20$; $p < 0.05$).

An analysis of variance based on forward step multiple regression is shown in table 4. Using Hb and SF <30 $\mu\text{g/l}$ as dependent variables, and based on all the predictor variables Hct and serum retinol were highly significant ($p < 0.01$) factors that explained 60 and 41% of the total variation in Hb status and SF concentrations, respectively. The correlation coefficients of Hct (0.42; 1.00) and serum retinol (1.81; 8.78), respectively for Hb status and SF concentrations show a positive direct linear relationship.

Backward multiple regression identified Hct ($p < 0.01$), breast milk fat ($p > 0.05$), serum retinol ($p < 0.05$), body mass index ($p < 0.05$), fat free mass ($p > 0.05$) and percent body fat ($p > 0.05$) as predictor variables able to explain 68% of the variation in the Hb status of the lactating women. Hct and serum retinol have a significant effect and need to be taken into account when making predictions and estimates that determine the Hb status and SF concentrations of lactating mothers.

With regard to maternal body composition, there may be a significant linear relationship between percent body fat, SF and retinol when you take into account maternal

age and the child's reported birth weight (table 5). Though these four factors explain 41% of the variation in percent body fat, except for maternal age, the other three coefficients show a negative direct linear relationship.

Discussion

The present study explores the relationship between 'serum retinol' and biochemical measures of iron nutrition and breast milk retinol, body composition in a population of lactating mothers in rural Kenya. A large percentage (73%) of the Kenyan population still lives in the rural area [15]. Nutritional studies in the country have tended to concentrate on the preschool-age child and to a very small degree on the pregnant mother. Lactating mothers were chosen as the subjects of this study because they are equally vulnerable to the documented vitamin A and iron metabolic interactions. During the reproductive cycle continuous depletion of these nutrients has negative effects on both the health and nutritional status of the mother and the infant. To this we now add the interactions likely to occur in relation to maternal body composition.

Table 5. Coefficients (β) and significance levels (p) from multiple regression analyses of percent body fat as the dependent variable for 88 lactating women

	Predictor variables			F	p
	β	r	r ²		
Constant	38.67				
Child birth weight	-3.52	0.34	0.12	5.48	<0.05
Constant	49.87				
Child birth weight	-5.18	0.47	0.22	5.64	<0.01
Serum retinol	-7.54				
Constant	51.54				
Child birth weight	-5.19	0.55	0.30	5.50	<0.01
Serum retinol	-6.91				
SF	-0.09				
Constant	41.55				
Child birth weight	-4.61	0.64	0.41	6.69	<0.01
Serum retinol	-6.56				
SF	-0.12				
Age of the mother	0.30				

The WHO identifies an Hb level below 12 g/dl as being anemic. We found 43.8% of the lactating mothers anemic by this criterion. This compares with the prevalence of 43% in nonpregnant women in the developing countries [25]. In a rural area in northern Natal a prevalence of anemia in females aged 6–74 years was found to be 52% [26]. In Bangladesh Ahmed et al. [27] found a prevalence of 44% among adolescent female garment factory workers with rural roots. When simple correlation tests were used we observed a highly significant correlation between Hb and Hct, SF concentration and MCV. This suggests that iron status was likely to be an important determinant of Hb and hence anemia. On the basis of combined cutoff points for Hb and SF (Hb < 12 g/dl and SF < 12 μ g/l) 27% of the lactating mothers with a mean Hb of 9.89 g/dl were iron-deficient. Similar relationships have been found in adolescent girls in peri-urban Bangladesh [28]. It was also found that those lactating mothers with depleted iron stores had significantly lower Hb and Hct compared with the lactating mothers who had SF levels \geq 12 μ g/l, reinforcing the impression that anemia in this group of lactating mothers was causally related to iron status. These mothers were also found to have lower serum retinol levels, indicating a possible relationship between vitamin A status and the use of iron for Hb formation.

A number of population studies have found positive correlations between serum retinol and biochemical indi-

cators of iron status [11, 12] and supplementation intervention has tended to target pregnant women and children. The results of our study show that even lactating mothers are equally at risk. The Hb and serum retinol relationship found in this study is similar to the one reported by Suharno [12] for pregnant women in West Java Indonesia. Bloem et al. [29] found that in children aged 1–8 years there was no association with Hb, but an association for serum retinol with transferrin and serum iron. In the lactating mothers studied, high levels of serum retinol were associated with higher levels of Hb ($r = 0.26$). Studies done in Bangladesh by Ahmed et al. [30] also showed that adolescent girls with higher Hb had better serum retinol levels.

Based on the WHO criteria for serum retinol and breast milk vitamin A concentration, the low levels of breast milk retinol and serum retinol identify this group of lactating mothers as being vitamin A-deficient [20]. A high percentage (88.4%) of lactating mothers were at risk of vitamin A deficiency with serum retinol values of less than 1.05 μ mol/l. The average breast milk fat concentration of 1.29 ± 0.69 μ mol/l is below the recommended cutoff value of 1.4 μ mol/l. In our study 78% of the infants were breast-fed on breast milk with an estimated vitamin A concentration of < 1.05 μ mol/l [20]. A recent analysis by Humphrey and Amy [3] based on data from studies conducted in Thailand by Stoltzfus and Underwood [22] suggests that this low intake puts the breast-fed infant at risk of vitamin A deficiency.

As shown in table 5, maternal fat stores during lactation need to take into account not only the negative effects on birth weight and maternal age but also on serum retinol and ferritin. The lactation phase of the reproductive cycle is energy-demanding. Maternal body composition stores are a proxy for adequacy of energy and protein intake. This in turn may also have an effect on the mother's iron and vitamin A status. There are no previous Kenyan studies that have looked at the relationship between body composition, vitamin A and iron status in lactating mothers. In this study, the occurrence of a negatively significant correlation between serum retinol and fat mass ($r = -0.040$) in mothers with a lactation period of < 4 months indicates a possible initial significant depletion in maternal energy stores.

With a prolonged lactation period of > 4 months the increase in breast milk fat is at the expense of a decrease in maternal protein reserves ($r = -0.27$). This may affect not only the concentration of breast milk vitamin A but also the long-term preservation of maternal energy and protein reserves [16]. The data show that in this rural Kenyan

community, mothers with a low level of fat mass also had a high prevalence of vitamin A and iron deficiency. Among rural Pakistan women maternal nutritional status was evaluated across a full reproductive cycle and it was found that marginally nourished women lost weight during the reproductive cycle concurrent with a positive trend in infant birth weight [31]. A study done on rural Bangladeshi women concludes that the women failed to gain sufficient weight during the last half of pregnancy to maintain body weight during lactation when energy demand is high [32]. Most studies on body composition during lactation have tended to concentrate on changes in body weight without taking into account the micronutrient deficiencies that are likely to occur. Ability to produce breast milk that is adequate to support the normal growth, nutrient stores and development of infants may be compromised when mothers consume poor-quality diets that are low in animal products and in most vitamins. Decades of exclusive support for children's programs have meant neglect of the welfare of the lactating mother. More work is needed to determine the optimal quantity of nutrients needed to replenish maternal stores and restore breast milk concentrations. In this study body composition indices were computed based on skinfold measurements. Use of more accurate methods may reveal a clearer picture of the extent of depletion in maternal energy and protein reserves.

The data show a high prevalence of nutritional anemia and subclinical vitamin A deficiency among these rural

lactating mothers. There is also an interaction between serum retinol, ferritin and percent body fat. In the present study we have shown that even in a group of lactating mothers who may appear otherwise well, marginal vitamin A status is likely to compromise iron stores. This makes it imperative to prevent maternal iron and vitamin A deficiency so that the true long-term benefits of these two nutrients are realized for both the mother and the infant. Mothers should be helped to enter their next pregnancy with better iron reserves [33, 34].

In summary, we found in this group of lactating women lower vitamin A and iron stores and that these indicators of malnutrition were related to each other. Changes in dietary intake may be the preferred long-term solution to the problem of maternal vitamin A and iron deficiency. There is a need to include lactating mothers in intervention strategies aimed at improvement of vitamin A and iron status.

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